Supporting Information

Theranostic Gold Nanoantennas for Simultaneous Multiplexed Raman Imaging of Immunomarkers and Photothermal Therapy

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Additional Figures and [MGNs] Equation:

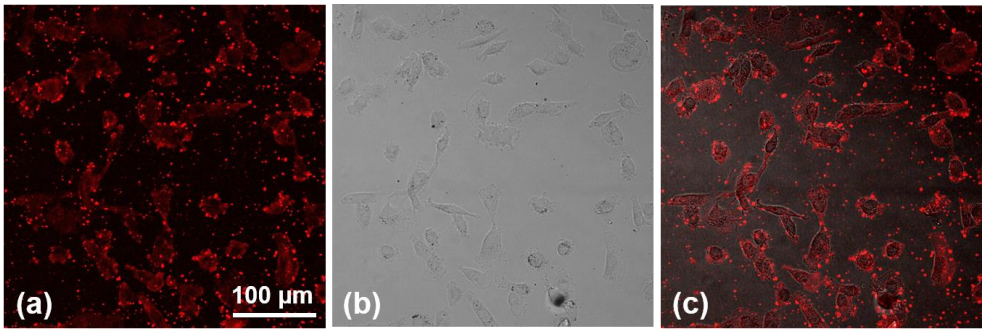


Figure S1. Fluorescent secondary antibody assay confirming Epidermal Growth Factor Receptor (EGFR) overexpression in MDA MB 231 cells. (a) Confocal fluorescent imaging of MDA-MB-231 cells after incubation with primary monoclonal antiEGFR, followed by a phycoerythin-labeled secondary antibody specific for the primary antiEGFR (excitation at 488 nm and emission 565-605 nm). (b) Brightfield images of the cells were also captured, and the (c) overlap was performed demonstrating surface binding.

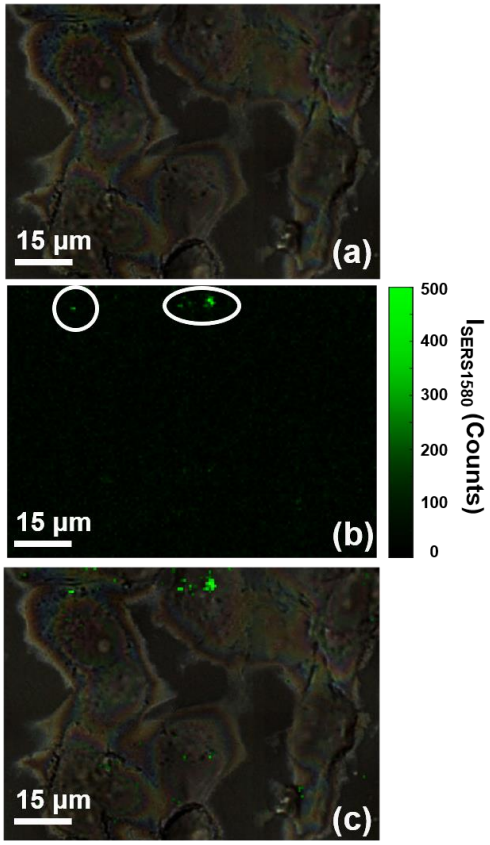


Figure S2. SERS mapping of MDA MB 231 cells with pMBA-MGNs. (a) Brightfield image of cells after 16 h incubation with pMBA-MGNs provides coordinates for Raman map. (b) Spatial Raman intensity map of the 1580 cm^{-1} peak corresponding to pMBA, with spectra recorded at 0.75 µm steps for the rectangular area in (a). (c) Overlap of (a) brightfield and (b) SERS map demonstrating low signal and minor surface binding of MGNs lacking targeting antibody.

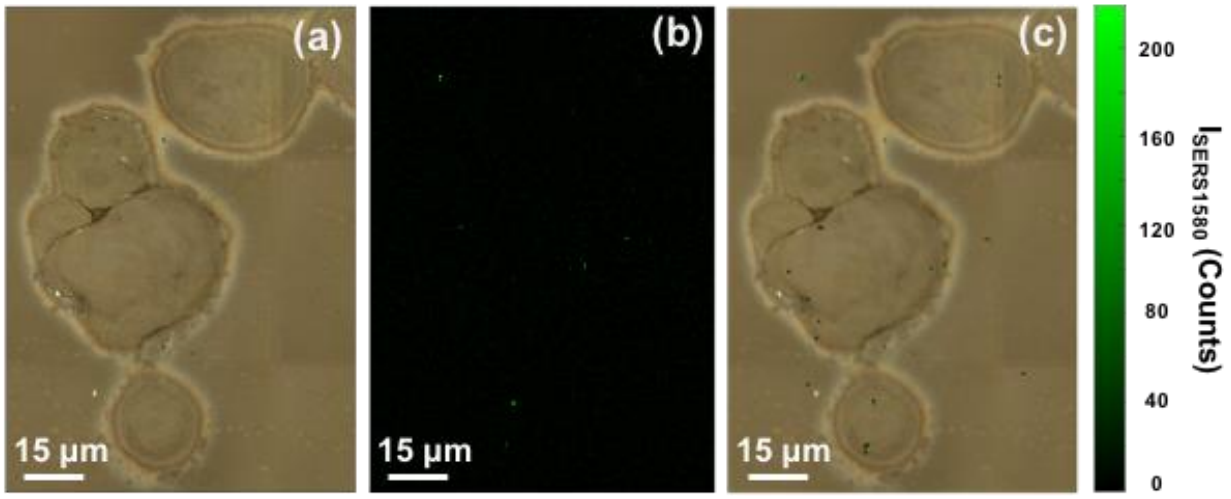


Figure S3. SERS singleplex mapping of MCF7 cells with antiEGFR-pMBA-MGNs. (a) Brightfield image of cells after 16 h incubation with antiEGFR-pMBA-MGNs provides coordinates for Raman map. (b) Spatial Raman intensity map of the 1580 cm^{-1} peak corresponding to pMBA, with spectra recorded at 0.75 µm steps for the rectangular area in (a). (c) Overlap of (a) brightfield and (b) SERS map demonstrating low signal and minimal surface binding of MGNs to MCF7 cells.

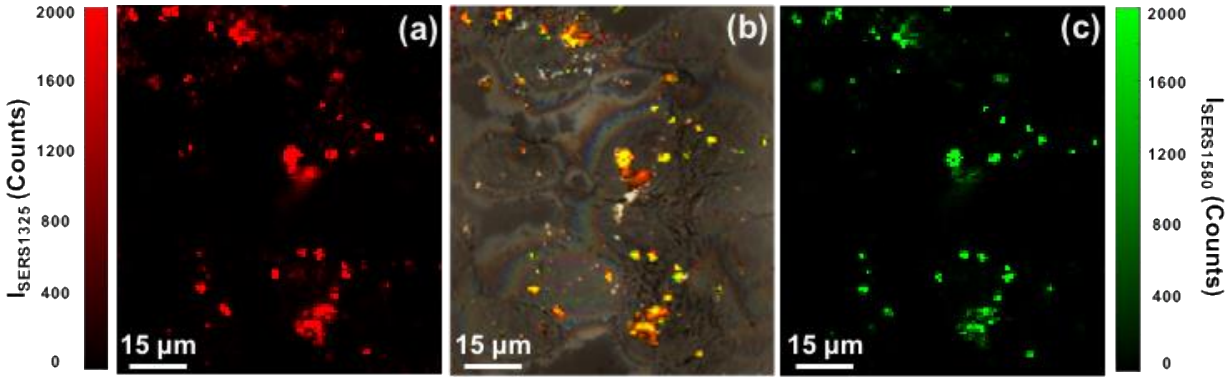


Figure S4. SERS multiplex mapping of MDA MB 231 cells with (1:1) mixture (antiEGFR-pMBA-MGNs to antiPDL1-DTNB-MGNs) additional plots. After 16 h incubation with (1:1) cocktail, spatial Raman intensity maps of (a) 1325 cm^{-1} peak for DTNB and (c) 1580 cm^{-1} peak for pMBA were recorded at 0.75 µm steps. Coordinates for map were provided by brightfield

image from Fig. 4a and the (b) overlap of “a” and “c” with the brightfield shows localization of the mixture of antibody labeled MGNs on the surface.

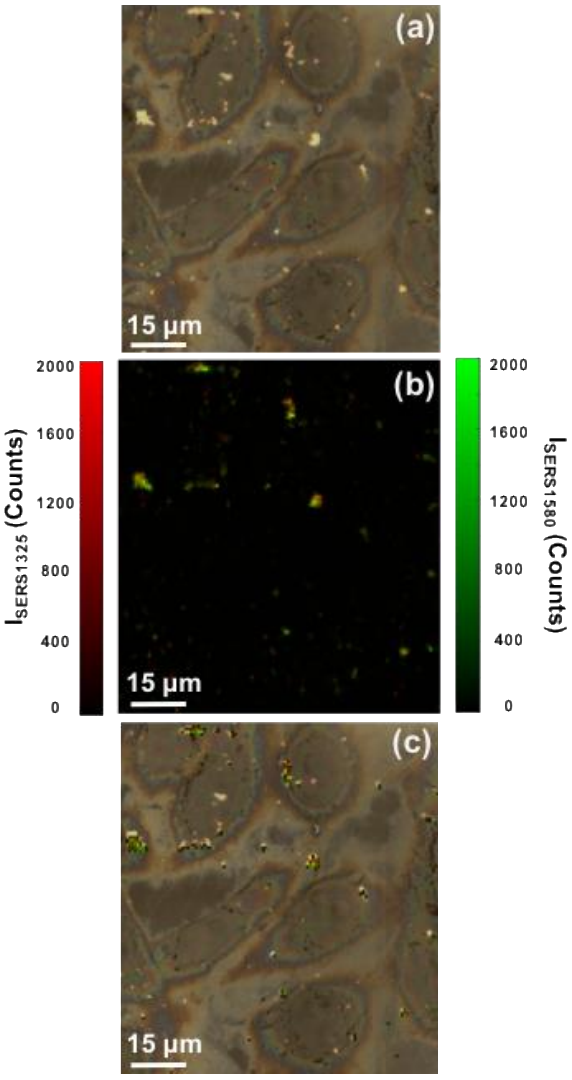


Figure S5. Blocking PD-L1 SERS multiplex mapping of MDA MB 231 cells with (1:1) mixture (antiEGFR-pMBA-MGNs to antiPDL1-DTNB-MGNs). Cells were pre-blocked with monoclonal antiPDL1 for 1 h. (a) Brightfield image of cells after 16h incubation with (1:1) mixture provides coordinates for multiplex Raman map. (b) Overlap of spatial Raman intensity maps of both $I_{SERS1325}$ for DTNB and $I_{SERS1580}$ for pMBA recorded at 0.75 µm steps. (c) Overlap of brightfield and SERS map (both $I_{SERS1325}$ and $I_{SERS1580}$) to compare the effects of blocking on surface binding.

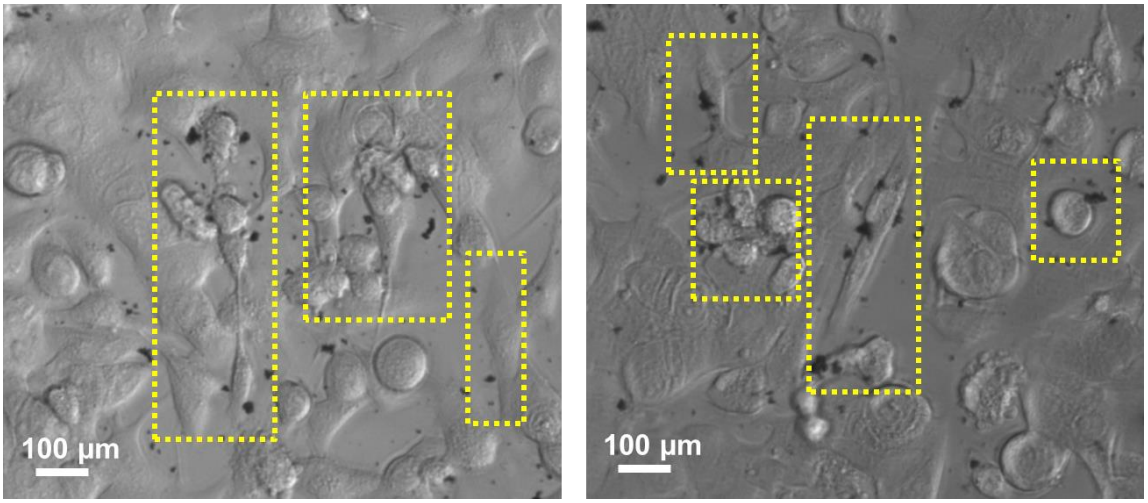


Figure S6. Live phase contrast images of MDA-MB-231 and MCF7 co-culture after 16 h of incubation with antiEGFR-pMBA-MGNs. Due to the distinct morphological differences in the two cell lines, they can be identified in the co-culture experiments. Spindle-like MDA-MB-231 cells are identified with yellow boxes showing MGNs binding whereas brick-shaped morphology of MCF7 shows minimal MGNs binding (MGNs are black spots).

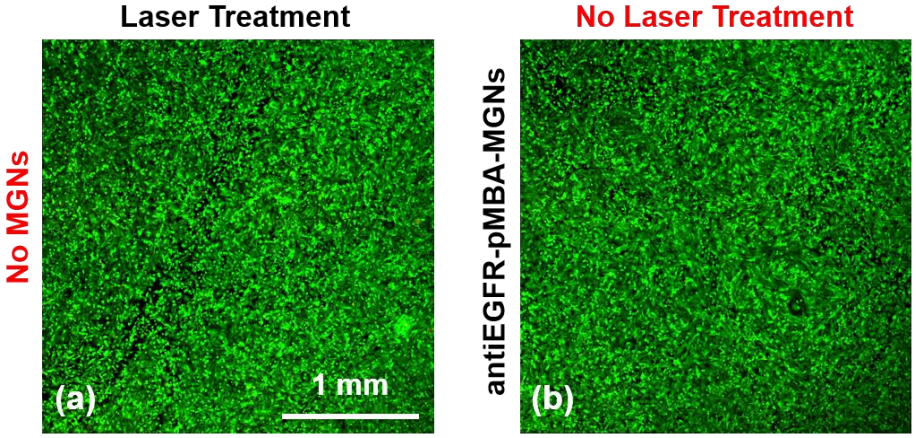
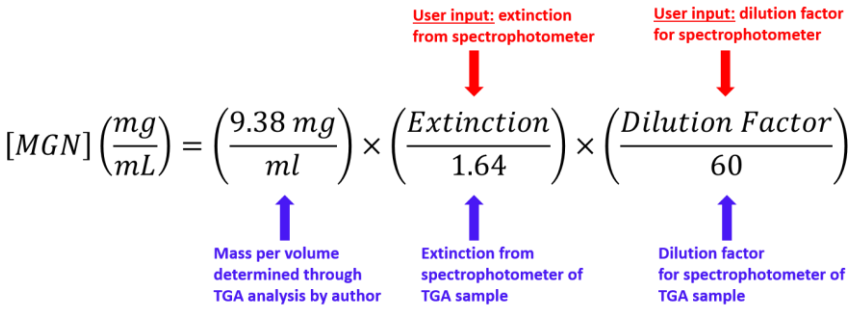


Figure S7. Photothermal therapy control assays, laser alone and antiEGFR-pMBA-MGNs alone. Confocal fluorescence images with live/dead cell stain (calcein AM/propidium iodide) showing no cell death in the MDA MB 231 cultures due to unwanted effects. (a) No observable cell death was detected when the 231 cells were illuminated in the absence of antiEGFR-pMBA-MGNs. (b)

Further the antiEGFR-pMBA-MGNs were not toxic to the cells following 16 h of incubation and repeated washing.



Equation S1. Equation for determining [MGNs]. Extinction spectra of MGNs was recorded with a 1 cm pathlength cuvette at a 60-fold dilution. A simultaneous thermogravimetric analyzer (TGA) study was performed on the same sample to quantify mass of MGNs per volume as demonstrated in previous work. Thus, equation was developed to calculate [MGNs] at various steps through the functionalization chemistry. User inputs values for synthesis and scaling.